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# THE DETERMINATION OF CHROMOSOMAL SEX BY ORAL SMEARS‡

The sex of an individual may be defined in three different ways:

a. The type of gonad present
b. General anatomical appearance and psychological orientation
c. Genetic factors as determined by the presence of the XX or XY chromosomes.

If uniformity is absent or equivocal in these characteristics, the decision as to whether an individual should be classified as male or female is difficult and is usually based on the prevailing general anatomical appearance. This may be unsatisfactory and is often misleading. General anatomical appearance and psychological orientation may be susceptible to spontaneous or iatrogenic changes. The chromosomal sex is perhaps closest to the "true" sex and is of greatest importance in the early recognition and treatment of intersexuality.

As early as 1917 Weiman,[20] and later Painter,[15] showed that sex chromosomes in the developing human male germ cell tend to remain compact with deep staining properties during the nuclear interphase. This is in contrast to other chromosomes which become so diffuse that it is difficult to identify them. Further, Geitler[7] demonstrated a sex difference in nuclear morphology in the *Gerris lateralis* and *Gerris lacustris,* waterbugs, on the basis of heterochromatic properties of their sex chromosomes. The early investigators in this field have therefore speculated that the fusion of the heterochromatic portions of the two X chromosomes in the female would form a mass large enough to be detected by ordinary microscopic means, the small size of the Y chromosome being probably the reason for the minute size of the sex chromatin in male nuclei. It was Barr *et al.* in 1950[2, 8, 13] who described for the first time an intranuclear body in the nerve cell of the cat, identified as the female sex chromatin. Large neurons seemed to be best suitable for this work because their nucleus is vesicular and lacks other

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chromatin particles. In these cells a chromatin mass about one micron in diameter was seen next to the nucleolus. This mass was encountered frequently in female cats but seldom occurred in the male.

The viscosity of the sex chromatin is such that, if free, it takes a round shape, while if adjacent to other structures, it is molded along the surface. If the nucleolus is eccentric, the chromatin is of discoid shape between the nucleolus and the membrane (Fig. 1), lying generally in the vicinity of the nucleolus. The staining qualities are those of desoxyribonucleic acid.

In 1953 Moore, Graham, and Barr[14, 1] devised a technique by which a chromosomal sex determination could be carried out from a skin biopsy in the human. Skin samples were stained by the Harris-Lena method (Gottenberg modification), and 100 cells were counted in the prickle cell layer and checked for the female chromatin. The incidence of this chromatin mass in cells of the skin originating from females was above 60 per cent, while specimens from males showed from 1 to 14 per cent incidence, a significant difference. Other authors have confirmed these findings.[6, 10, 11] Studies on the sex chromatin in other tissues have been published since. Davidson and Smith[5] reported on chromosomal sex determination in neutrophiles from the peripheral blood. Sohval and Gaines[18] have studied the sexual differences in nuclear morphology of tumors, inflammations, and squamous metaplasia. They found no discrepancy in the chromosomal sex of host and tumor except in the instance of a teratomatous growth (see also Cruikshank[4]). Shettles[17] was able to predict fetal sex from squamous cells recovered from amniotic fluid during labor. Moore and Barr,[12] as well as Marberger,[10] have achieved satisfactory results using a mucosal smear stained with a modified Feulgen or cresyl violet stain.

The main application of this new diagnostic tool is in the study of the true and pseudohermaphrodite, especially in early age when hormone studies are difficult to obtain or are not conclusive.

At six to eight weeks in the human embryo the genitalia differentiate from a Wolffian or Mullerian duct system, both of which are present in early embryonic life. It seems to be well established by the work of Jost, Raynard, and Wells that the stimuli for differentiation of the genitalia are provided by the gonads. These authors were able to destroy *in utero* the gonadal anlage before differentiation of the gonad in mice, rats, and rabbits. The fetuses all showed development of the female external genitalia only. The investigators therefore believe that the male primary sex characteristics depend upon stimulation from the gonad. This theory may partially explain why presumably female patients with ovarian agenesis (or better, gonadal dysgenesis) have been reported as chromosomal males.[3, 9, 19, 16] The fact that approximately 80 per cent instead of 50 per cent of these lack the

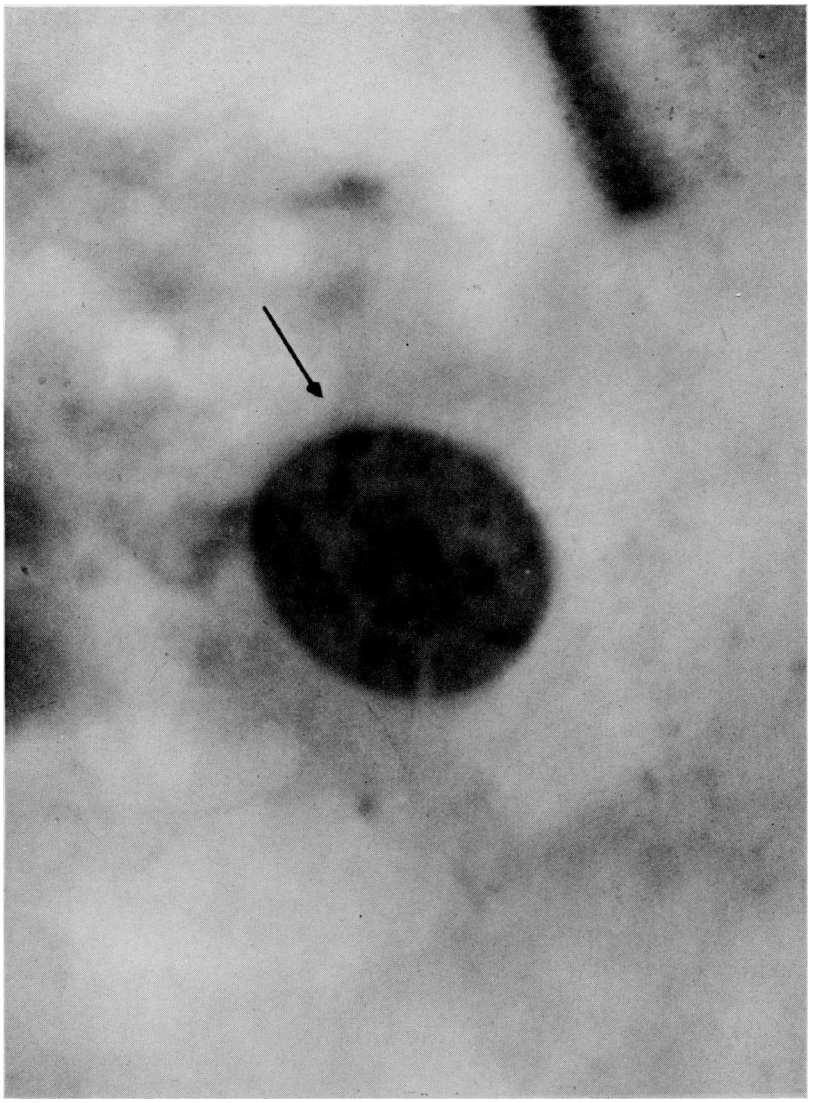

FIG. 1. Sex chromatin in an epithelial cell from the oral mucosa.

female sex chromatin is as yet unexplained. The cause of gonadal agenesis in these cases has not been explained either, but may possibly be attributed to chromosomal aberration or damage.

While the skin biopsy gives very satisfactory results, a simpler and less traumatic method seemed to be desirable. We have therefore analyzed some

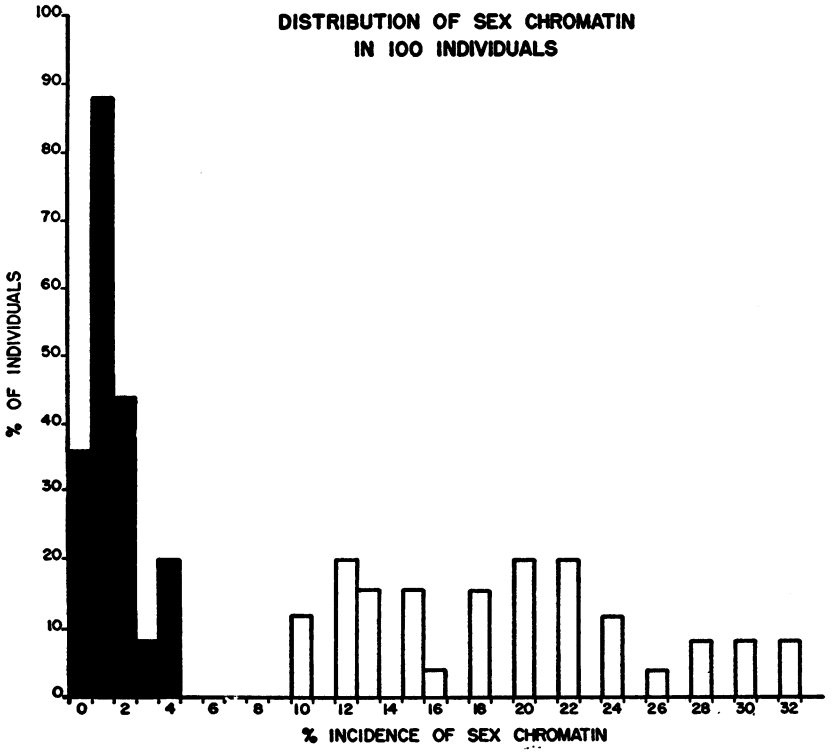


FIG. 2. Distribution of sex chromatin in 100 individuals.

of our routine vaginal smears and found that they also contained the sex chromatin. Oral smears were then done on 100 persons of varying ages for a control series. Fifty males and 50 females each had the buccal mucosa scraped with a wooden spatula. The material obtained was smeared on a glass slide and immediately dropped in a solution containing equal parts of ether and 95 per cent ethyl alcohol. The slides were then stained with the Papanicolaou stain. This stain was used because it had been determined previously that it was satisfactory for identification of the sex component. Each slide was studied under oil immersion lens, and the sex chromatin

was easily identified. The sex of the person from whom the specimen was obtained was not known until after the counts had been made. One hundred cells of the parabasal and precornified type were counted and the percentage of cells containing this component determined. In the control series proper

TABLE 1.

| *Clinical diagnosis* | *Anatomical sex* | *Incidence of female sex chromatin* | *Chromosomal sex* |
|---|---|---|---|
| Pseudohermaphrodite (Adrenal hyperplasia) | female | 15% | female |
| Pseudohermaphrodite (?Adrenal hyperplasia) | female | 12% | female |
| Pseudohermaphrodite (?Adrenal hyperplasia) | female | 22% | female |
| Pseudohermaphrodite (Adrenal hyperplasia) | male | 14% | female |
| Gonadal dysgenesis | female | 1% | male |
| Gonadal dysgenesis | female | 1% | male |
| Gonadal dysgenesis | female | 10% | female |
| Gonadal dysgenesis | female | 11% | female |
| Gonadal dysgenesis | female | 10% | female |
| "Behaviour problem" | male | 1% | male |
| True hermaphrodite | female | 34% | female |
| Primary amenorrhea | female | 16% | female |
| Absence of vagina | female | 16% | female |
| Testicular agenesis | male | 4% | male |
| Multiple congenital malformations | female | 48% | female |

identification of the sex of the individual was made on the first count on all 100 slides.

As illustrated by Figure 2, there were two definite groups, one containing from 0-4 per cent and the other from 10-32 per cent incidence of sex chromatin respectively. The first group included all the males, while all of the females studied were found in the second group. Furthermore, 84 per cent of the males had an incidence of the sex chromatin of 0-2 per cent, while the females of this control group were rather uniformly distributed between 10 and 32 per cent.

Since this test for sex differentiation has proved so accurate, a decision was made to apply it to those cases where clinical evaluation of sex was difficult or questionable. Fifteen patients of this type have been examined so far with interesting and sometimes unexpected results.

As will be seen from Table 1, four of these cases were clinically diagnosed as pseudohermaphrodites, three being considered anatomically female and one anatomically male. One of the three females was a proven case of congenital adrenal hyperplasia, diagnosis confirmed by the elevated beta fraction of the 17-ketosteroids. The other two were newborn twins on whom no endocrine essays have yet been performed. These twins showed a phallus-like development of the clitoris. All three cases had a female chromosomal pattern. The anatomically male pseudohermaphrodite was 44 years old and had been previously diagnosed as a male achondroplastic dwarf. He had a phallus measuring 3½ inches with hypospadias and a bifid scrotum. No testes were palpable. Steroid studies proved this case also to be a congenital adrenal hyperplasia. A chromatin count of 14 per cent indicated that this patient was genetically female.

Two of the gonadal dysgenesis cases proved to be chromosomally male even though they had female genitalia and were living satisfactory lives emotionally, physically, and mentally as females. This is in accord with the findings of Wilkins[21] and others. The true hermaphrodite had female external genitalia, a testis on one side and an ovary on the other, the chromatin count followed a female pattern. In the remaining cases the chromosomal sex corresponded with the anatomical sex.

In addition to its potentialities in genetic investigation this test has a very real clinical value. This is especially so in assisting the clinically sexually indistinguishable individual toward proper sexual orientation.

## CONCLUSIONS

1. A variation of the Barr technique of chromosomal sex differentiation is reported.
2. Its accuracy has been verified on 100 control cases.
3. Its use in problems of clinical sex differentiation is discussed, 15 such cases being reported.

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